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ON THE EXISTENCE AND UNIQUENESS OF BIOLOGICAL CLOCK MODELS MATCHING EXPERIMENTAL DATA*

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Abstract. The development of luciferase markers and other experimental techniques has allowed measurement of the timecourses of the expression of genes and proteins with remarkable accuracy. Since these data have been used to develop many mathematical models, it is important to ask whether this problem of model building is well-posed. Here, we focus on a common form of ordinary differential equation (ODE) models for biological clocks, which consist of production and degradation terms, and assume we have an accurate measurement of their solution. Given these solutions, do ODE models exist? If they exist, are they unique? We show that timecourse data can sometimes, but not always, determine the unique quantitative relationships (i.e., biochemical rates) of network species. In other cases, our techniques can rule out functional relationships between network components and show how timecourses can reveal the underlying network structure. We also show that another class of models is guaranteed to have existence and uniqueness, although its biological application is less clear. Our work shows how the mathematical analysis of the process of model building is an important part of the study of mathematical models of biological clocks.

Key words. biological clock models, timecourses, existence and uniqueness, iterative maps, inverse problems, periodic solutions, molecular networks

AMS subject classifications. 34A12, 34A55, 34C25, 92B05, 92B25, 92C42

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1. Introduction.

A tremendous amount of data has become available on biological clocks during the past 15 years. Most of this data comes in the form of timecourses, i.e., measurements over time of the concentrations of chemical species with GFP (green fluorescent protein), luciferase, or microarray techniques. Many mathematical modeling studies have used timecourse data to estimate the parameters of their models (e.g., [4], [13]). This raises the question of whether timecourses can do more than the parameter estimation. That is, can timecourses reveal information about network structure?

We answer this question with a common form of ordinary differential equation (ODE) models for biological clocks [6], [7], [15],

$$\frac{dr}{dt} = f(s) - g(r), \tag{1.1}$$

in which $f(s)$ represents the production of r based on some other species, s is the network, and $g(r)$ is the rate of clearance of r. Here, we consider the following questions, given precise measurements of two oscillating timecourses $r(t)$ and $s(t)$ with the same period:

Question 1 (*existence*). Can we find f and g such that the solution of (1.1) matches the given timecourses $r(t)$ and $s(t)$?

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Question 2 (*uniqueness*). If we can find f and g, are they unique?

The existence of f and g implies that the production of r is controlled by s. The uniqueness of f and g implies that the timecourses are enough to determine the quantitative relationships (i.e., biochemical rates).

The questions we ask here are related to several other well-studied problems.

(1) Control theorists consider a related problem called "identifiability" [12], [16]. This problem usually focuses on the identification of parameters of a given ODE with control of inputs and measurable outputs (e.g., timecourses). Here, we look for functions of ODEs rather than parameters of ODEs matching timecourses without control of input.

(2) Symbolic dynamics and reverse engineering techniques have been used to determine the qualitative functional relationships between species (i.e., whether activation or repression occurred) [2], [8], [14], [17]. Here, we pursue more detailed information. For instance, we ask whether the timecourses contain information for the quantitative relationship (e.g., determining f and g) as well as qualitative relationships.

(3) Recently, harmonic balance techniques have been proposed to select an ODE model from a class of models based on the best match to given oscillating timecourses [5], [9]. However, here we search for the models matching timecourses exactly, instead of selecting an ODE matching timecourses better than the other candidates.

(4) One of the classical results in the theory of ODEs is that, given a sufficiently smooth ODE and an initial condition, a unique solution exists [11]. Here, we ask the opposite question: Given the oscillating solution of an ODE, can we find an ODE matching this solution, and is this ODE unique?

(5) Chemical reaction network theory has shown that the same ODE system may result from different reaction network diagrams [1]. Here, we study the relationship between the ODE model and timecourses rather than that between the ODE model and reaction network. Paper [1] often equates dynamics (i.e., timecourses of key chemical species) with an ODE model. However, in practical applications, an ODE model is rarely known, and almost never known fully. Thus, it is important to know how the timecourses determine ODE dynamics.

Our main finding is a necessary condition for Question 1 that provides a tool for detecting the relationship among the species solely from their timecourses. We also find necessary and sufficient conditions for Question 2, when timecourse data is enough to determine an ODE model of the form (1.1) uniquely. Finally, we show that another class of models of the form (1.2) (previously proposed in [3]), as well as the class of models of the form (1.3), have uniqueness and existence over a broad class of timecourses:

$$\frac{df(r)}{dt} = s + g(r), \tag{1.2}$$

$$\frac{dr}{dt} = f(r)s - g(r). \tag{1.3}$$

This paper is organized in the following way. In section 2, we outline our results. In section 3, we present the necessary condition for existence of (1.1) and examples to show how it can reveal the structure from timecourse data. In section 4, we present the necessary and sufficient conditions for uniqueness of (1.1). Section 5 demonstrates that existence and uniqueness hold for (1.2) and (1.3).

2. Overview of existence and uniqueness of models.

Definition 2.1. *$r(t)$ and $s(t)$ are smooth oscillating timecourses with period τ (τ-periodic) and with one maximum and one minimum per period.*

In this paper, we consider only smooth (C^∞) functions because most biological problems are smooth. However, most of the results still hold when this assumption is relaxed.

Definition 2.2. *If $k(t)$ is a smooth timecourse with period τ and with one maximum and one minimum over a period, then $\hat{k}(t) : [-\tau/2, \tau/2) \to [-\tau/2, \tau/2)$ is a one to one function such that $k(\hat{k}(t)) = k(t)$ and $\hat{k}(t) \neq t$ unless t is a maximum or minimum of $k(t)$.*

For example,

$$\widehat{\cos}(2\pi t/\tau) = \begin{cases} -t & \text{if } -\tau/2 < t < \tau/2, \\ -\tau/2 & \text{if } t = -\tau/2 \end{cases}$$

and

$$\widehat{\sin}(2\pi t/\tau) = \begin{cases} -t + \tau/2 & \text{if } 0 < t < \tau/2, \\ -t - \tau/2 & \text{if } -\tau/2 \leq t \leq 0. \end{cases}$$

Definition 2.3. *Given $r(t)$ and $s(t)$, $\phi(t) := \hat{s}(\hat{r}(t))$.*

Our results are described in Figure 2.1. The fixed points of $\phi(t)$ and its iteration $\phi^n(t)$ play a pivotal role in determining the existence and uniqueness of models given timecourses $r(t)$ and $s(t)$. Theorem 3.1 shows that the existence of fixed points satisfying (3.2) implies the nonexistence of (1.1). Theorem 4.3 shows that a model of the form (1.1) is unique as long as the set of fixed points is of measure zero. Theorem 5.2 shows the existence and uniqueness of (1.2) for a broad class of timecourses. Theorem 5.3 shows that (1.2) can be converted into a more canonical form (1.3) if $\phi(t)$ does not have a fixed point.

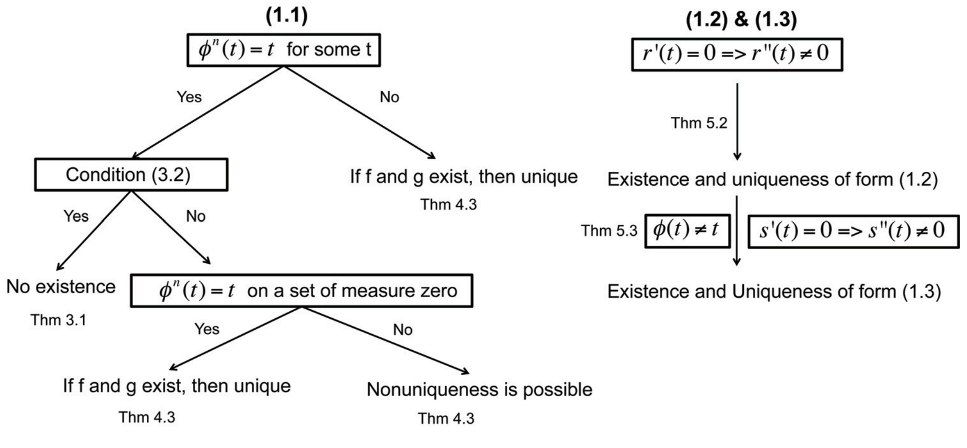


Fig. 2.1. *Existence and uniqueness of f and g of forms* (1.1), (1.2), *and* (1.3) *matching timecourses $r(t)$ and $s(t)$.*

3. A necessary condition for the existence of (1.1).

THEOREM 3.1. *If there exist a* $\hat{t} \in [-\tau/2, \tau/2)$ *and an* $n \in N$ *for* $r(t)$ *and* $s(t)$ *such that*

$$\phi^n(\hat{t}) = \hat{t}, \tag{3.1}$$

$$\frac{dr}{dt}_{t=\hat{t}} + \frac{dr}{dt}_{t=\phi(\hat{t})} + \cdots + \frac{dr}{dt}_{t=\phi^{n-1}(\hat{t})} \neq \frac{dr}{dt}_{t=\hat{r}(\hat{t})} + \frac{dr}{dt}_{t=\hat{r}(\phi(\hat{t}))} + \cdots + \frac{dr}{dt}_{t=\hat{r}(\phi^{n-1}(\hat{t}))}, \tag{3.2}$$

then functions f *and* g *of form* (1.1) *do not exist.*

Proof. This can be proved by showing that if there exist f and g satisfying (1.1), then (3.1) implies the equality in (3.2).

Equation (3.1) implies that

$$\hat{t} = t_0 \xrightarrow{\hat{r}} t_1 \xrightarrow{\hat{s}} t_2 \xrightarrow{\hat{r}} t_3 \cdots \xrightarrow{\hat{s}} t_{2n-2} \xrightarrow{\hat{r}} t_{2n-1} \xrightarrow{\hat{s}} t_0 = \hat{t}. \tag{3.3}$$

Since $\hat{r}^{-1}(t) = \hat{r}(t)$ and $\hat{s}^{-1}(t) = \hat{s}(t)$,

$$\hat{t} = t_0 \xleftarrow{\hat{r}} t_1 \xleftarrow{\hat{s}} t_2 \xleftarrow{\hat{r}} t_3 \cdots \xleftarrow{\hat{s}} t_{2n-2} \xleftarrow{\hat{r}} t_{2n-1} \xleftarrow{\hat{s}} t_0 = \hat{t}. \tag{3.4}$$

Let us show that (3.1) implies the equality in (3.2). If we assume that there exist f and g satisfying (1.1), then

$$\begin{aligned}
&\frac{dr}{dt}_{t=\hat{t}} + \frac{dr}{dt}_{t=\phi(\hat{t})} + \cdots + \frac{dr}{dt}_{t=\phi^{n-1}(\hat{t})} \\
&\quad = f(s(\hat{t})) - g(r(\hat{t})) + f(s(\phi(\hat{t}))) - g(r(\phi(\hat{t}))) + \cdots + f(s(\phi^{n-1}(\hat{t}))) - g(r(\phi^{n-1}(\hat{t}))) \\
&\quad = f(s(t_0)) - g(r(t_0)) + f(s(t_2)) - g(r(t_2)) + \cdots + f(s(t_{2n-2})) - g(r(t_{2n-2})) \\
&\quad = f(s(t_0)) + f(s(t_2)) + \cdots + f(s(t_{2n-2})) - g(r(t_0)) - g(r(t_2)) - \cdots - g(r(t_{2n-2})).
\end{aligned} \tag{3.5}$$

Since $r(\hat{r}(t)) = r(t)$, $s(\hat{s}(t)) = s(t)$, and (3.4) holds,

$$\begin{aligned}
&f(s(t_0)) + f(s(t_2)) + \cdots + f(s(t_{2n-2})) - g(r(t_0)) - g(r(t_2)) - \cdots - g(r(t_{2n-2})) \\
&\quad = f(s(\hat{s}(t_0))) + f(s(\hat{s}(t_2))) + \cdots + f(s(\hat{s}(t_{2n-2}))) - g(r(\hat{r}(t_0))) - g(r(\hat{r}(t_2))) - \\
&\qquad \cdots - g(r(\hat{r}(t_{2n-2}))) \\
&\quad = f(s(t_{2n-1})) + f(s(t_1)) + \cdots + f(s(t_{2n-3})) - g(r(t_1)) - g(r(t_3)) - \cdots - g(r(t_{2n-1})) \\
&\quad = f(s(t_1)) - g(r(t_1)) + f(s(t_3)) - g(r(t_3)) + \cdots + f(s(t_{2n-1})) - g(r(t_{2n-1})) \\
&\quad = r'(t_1) + r'(t_3) + \cdots + r'(t_{2n-1}) \\
&\quad = \frac{dr}{dt}_{t=\hat{r}(\hat{t})} + \frac{dr}{dt}_{t=\hat{r}(\phi(\hat{t}))} + \cdots + \frac{dr}{dt}_{t=\hat{r}(\phi^{n-1}(\hat{t}))}.
\end{aligned} \tag{3.6}$$

Equations (3.5) and (3.6) imply that if there exist f and g satisfying (1.1), then (3.1) contradicts (3.2). Therefore, conditions (3.1) and (3.2) imply that f and g of (1.1) do not exist. ∎

A simple example to illustrate Theorem 3.1 is provided in Figure 3.1. Since $\phi(t_0) = \hat{s}(\hat{r}(t_0)) = \hat{s}(t_1) = t_0$, both $r(t)$ and $s(t)$ have the same value at t_0 and t_1, so both $g(r(t))$ and $f(s(t))$ do as well. This implies the equality of (3.2) or $r'(t_0) = r'(t_1)$

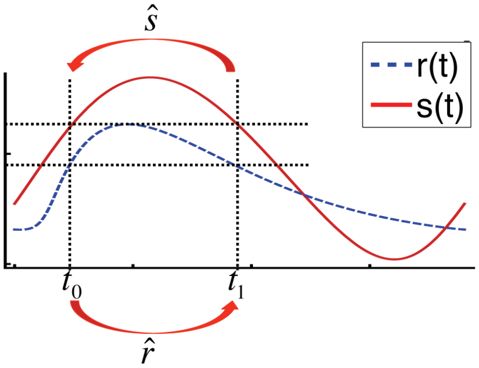


FIG. 3.1. *Fixed point of* $\phi(t)$ *implies the nonexistence of the model* (1.1).

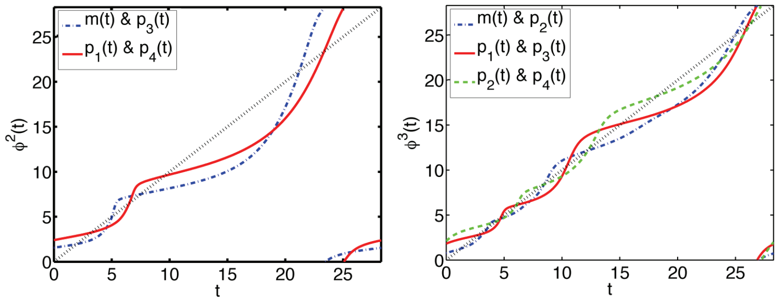
if there exists a model (1.1). However, $r'(t_0) \neq r'(t_1)$ unless $r'(t_0) = 0$ and $r'(t_1) = 0$ since the signs of $r'(t_0)$ and $r'(t_1)$ are opposite (Figure 3.1). Therefore, the condition (3.1) implies the nonexistence of the model (1.1) unless $r'(t_0) = 0$ and $r'(t_1) = 0$.

Theorem 3.1 provides a way to determine the structure of the molecular network by showing the nonexistence of (1.1) among unrelated species. Let us apply this to reveal the structure of biological clock model (3.7) based on the Goodwin oscillator [6], [7], [15]:

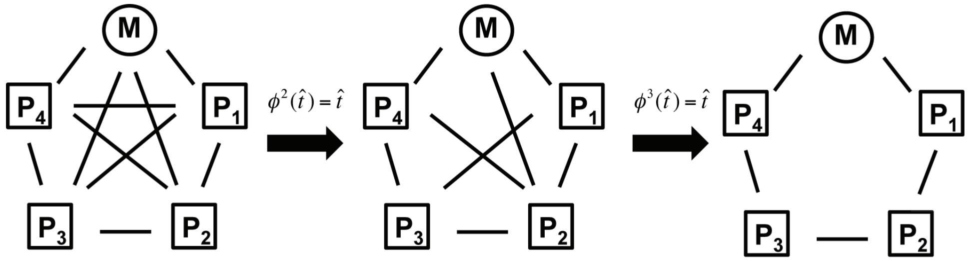
$$\begin{aligned}
\frac{dM}{dt} &= \frac{1}{1+P_4^{10}} - 0.4M,\\
\frac{dP_1}{dt} &= M - 0.4P_1,\\
\frac{dP_2}{dt} &= P_1 - 0.4P_2,\\
\frac{dP_3}{dt} &= P_2 - 0.4P_3,\\
\frac{dP_4}{dt} &= P_3 - 0.4P_4.
\end{aligned} \tag{3.7}$$

Simulating this model generates 5 timecourses, which are interrelated in a single negative feedback loop. From these timecourses, we seek to reconstruct the model's structure, without knowledge of (3.7). Given the 5 timecourses, 10 interactions are possible of the form given in (1.1) (see Figure 3.2(c)). Can Theorem 3.1 be used to find the relevant interactions? To determine whether two components of the system are related via (1.1) with Theorem 3.1, we check whether there exist some t obeying (3.1) and at that t, (3.2) is also satisfied. As shown in Figure 3.2(a), we find that there exists t such that $\phi^2(t) = t$ as well as (3.2) are satisfied between the pair $m(t)$ and $p_3(t)$ and the pair $p_1(t)$ and $p_4(t)$. Thus, we can rule out these interactions. In a similar way, by checking $\phi^3(t) = t$ and (3.2), three more interactions are excluded: $m(t)$ and $p_2(t)$, $p_1(t)$ and $p_3(t)$, and $p_2(t)$ and $p_4(t)$ (Figure 3.2(b)). The only remaining interactions now match the original feedback loop structure (Figure 3.2(c)). Thus from the timecourse data and Theorem 3.1, we were able to reconstruct the original feedback loop.

We also apply Theorem 3.1 to another problem to determine whether it can be used to distinguish between two independent Goodwin oscillators. For this, we



(a) $\phi^2(t) = t$. (b) $\phi^3(t) = t$.



(c) Reconstruction of (3.7).

FIG. 3.2. *Theorem* 3.1 *excludes unrelated pair of signals of* (3.7), *so the original feedback loop structure is identified from the timecourses.*

construct two Goodwin oscillators with different Hill-coefficients, (3.8) and (3.9). By choosing $ts = 1.356$ in all the reactions of (3.9), we can match the periods of (3.8) and (3.9):

$$
\begin{aligned}
\frac{dM}{dt} &= \frac{1}{1+P_2^9} - 0.1M,\\
\frac{dP_1}{dt} &= M - 0.1P_1,\\
\frac{dP_2}{dt} &= P_1 - 0.1P_2,
\end{aligned}
\tag{3.8}
$$

$$
\begin{aligned}
\frac{dN}{dt} &= ts\left(\frac{1}{1+Q_2^{20}} - 0.1N\right),\\
\frac{dQ_1}{dt} &= ts(N - 0.1Q_1),\\
\frac{dQ_2}{dt} &= ts(Q_1 - 0.1Q_2).
\end{aligned}
\tag{3.9}
$$

There are 15 possible interacting pairs between the 6 variables of (3.8) and (3.9) (e.g., Q_1 and M, and P_1 and P_2) (see Figure 3.3). For 2 pairs of variables, there exists a t such that $\phi(t) = t$ as well as (3.2), which means they are not related. By checking $\phi^2(t) = t$, 4 more pairs are excluded. $\phi^3(t) = t$ excludes 1 more pair. Now, 7 pairs among the original 15 pairs are excluded. By comparing them with (3.8) and (3.9), we see that 7 of the 9 unrelated pairs are correctly excluded. This shows how Theorem

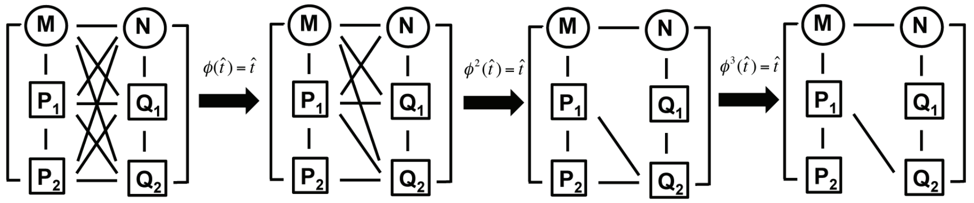


FIG. 3.3. *Theorem* 3.1 *excludes most unrelated pairs of components in two independent Goodwin oscillators,* (3.8) *and* (3.9). *(Initial condition used:* $M(0) = 0.0227$*,* $P_1(0) = 0.1472$*,* $P_2(0) = 1.7531$*,* $N(0) = 0.0034$*,* $Q_1(0) = 0.1269$*, and* $Q_2(0) = 2.4147$*).*

3.1 can be used to identify incorrect functional relationships between variables and reveal the structure.

Theorem 3.1 considers a simple model (1.1), in which each component is regulated by only one other component. However, in real biological systems, this assumption may be too simplistic. If more than one component $s_1, s_2, \dots, s_n$ controls the production of a chemical species, we have the following form:

$$\frac{dr}{dt} = f(s_1, s_2, \dots, s_n) - g(r).$$

One particular case of this is

$$\frac{dr}{dt} = f_1(s_1) + f_2(s_2) - g(r),$$

where we have more freedom to choose f_1 and f_2 than if we can choose only one f as in (1.1). Therefore, we can expect that the existence is more likely to occur as more species or variables are included in the model. Since $\phi(t) = \hat{s}(\hat{r}(t))$ plays a key role in the study of (1.1), similar techniques could be used to study the generalized models by finding a new version of $\phi(t) = \hat{s}(\hat{r}(t))$, which describes the relationship of more than two timecourses. It may also be helpful to experimentally keep some of the signals (e.g., $s_2, s_3, \dots$) constant, which would reduce the problem to that of the form (1.1).

4. Necessary and sufficient conditions for uniqueness of (1.1).

Given two timecourses, for which an appropriate f and g of (1.1) can be found, we next consider whether the functions f and g are unique. One trivial way to achieve nonuniqueness is to add the same constant to both f and g since we are concerned only with their difference in the ODE (1.1). To avoid this, we assume that $f(0) = 0$. However, as shown by the following theorem, this alone does not guarantee uniqueness.

THEOREM 4.1. *Given two timecourses $r(t)$ and $s(t)$ for which f and g of* (1.1) *exist, smooth functions f and g are unique if and only if there are no smooth functions m and n such that*

$$m(s(t)) = n(r(t)) \text{ and } m(0) = 0. \tag{4.1}$$

Proof (only if). Let us assume that there are f_1, g_1, f_2, and g_2 such that

$$\frac{dr}{dt} = f_1(s) - g_1(r) = f_2(s) - g_2(r).$$

By defining,

$$m(s) := f_1(s) - f_2(s),$$
$$n(r) := g_1(r) - g_2(r),$$

we show that $m(s) = n(r)$ and $m(0) = f_1(0) - f_2(0) = 0 - 0 = 0$.

(If). Now, let us assume that there exist m and n such that $m(s) = n(r)$ and $m(0) = 0$ and show that f and g of (1.1) are not unique. Then,

$$\frac{dr}{dt} = f(s) - g(r) = f(s) + m(s) - n(r) - g(r).$$

Let

$$\begin{aligned} \tilde{f}(s) &:= f(s) + m(s), \\ \tilde{g}(r) &:= g(r) + n(r). \end{aligned} \tag{4.2}$$

Then, $r(t)$ and $s(t)$ become solutions of (1.1) with these new functions and $\tilde{f}(0) = f(0) + m(0) = 0 + 0 = 0$. $\square$

The class of functions $s(t)$ and $r(t)$, which do not have uniqueness, is not apparent immediately since determining the existence of m and n such that $m(s) = n(r)$ is difficult, especially when nonmonotonic m and n are allowed. The following corollary is useful in seeing how large the space of $s(t)$ and $r(t)$ with nonunique f and g is.

Corollary 4.2. *Given two timecourses $r(t)$ and $s(t)$, for which f and g of* (1.1) *exist, smooth functions f and g are unique if and only if there is no smooth function m such that*

$$m(s(-t')) = m(s(t')) \text{ and } m(0) = 0 \tag{4.3}$$

for the scaling of time $t'(t)$, with which $r(t')$ is an even function.

Proof. We can shift time so that $r(t)$ has a maximum at $t = 0$. Then, we can always find the scaling of time $t'(t)$, with which $r(t')$ becomes an even function (see [3] for details). Then, $n(r(t'))$ of (4.1) also becomes an even function. This causes the nonuniqueness condition (4.1) with respect to the scaling of time $t'(t)$ to be that $m(s(t'))$ is an even function. $\square$

This corollary shows that if f and g of (1.1) exist given $r(t)$ and $s(t)$, then f and g are not unique when $s(t)$ is in the preimage of an oscillating even function with time scaled so that $r(t)$ becomes an even function. The preimage of oscillating even functions is broad and contains even functions, odd functions, simple harmonic functions $\sin(t + m\pi/n)$, and so on. At first glance, nonuniqueness seems to frequently occur because, regardless of what $r(t)$ is, there always exists a large space (the preimage of oscillating even functions in the new scaling of time) of $s(t)$, in which nonunique f and g of (1.1) exist. However, the following theorem shows that nonuniqueness is less likely to occur by showing that an $s(t)$ which is the preimage of oscillating even function cannot be a solution of (1.1) in most cases.

Theorem 4.3. *Given two timecourses $r(t)$ and $s(t)$, for which f and g of* (1.1) *exist, f and g of form* (1.1) *are unique if and only if $\phi^n(t) \neq t$ for almost every t and for all $n \in N$.*

Proof (if). Let us assume that there exist nonunique f and g of (1.1) or that $m(s(t'))$ is an even function for the scaling of time $t'(t)$ for which $r(t')$ is an even function by Corollary 4.2. Since $m(s(t'))$ is an even function and $\hat{r}(t') = -t'$ on $(-\tau/2, \tau/2)$,

$$m(s(t')) = m(s(-t')) = m(s(\hat{r}(t'))). \tag{4.4}$$

Since $s(\hat{r}(t')) = s(\hat{s}(\hat{r}(t')))$ by definition of $\hat{s}(t)$,

$$m(s(\hat{r}(t'))) = m(s(\hat{s}(\hat{r}(t')))) = m(s(\phi(t'))). \tag{4.5}$$

Therefore, (4.4) and (4.5) imply that $m(s(t'))$ has the same value for t' and $\phi(t')$. By applying the same argument for $\phi(t')$ instead of t', we can show that $m(s(t'))$ has the same value for the following set:

$$\{t', \phi(t'), \phi^2(t'), \ldots, \phi^n(t'), \ldots\}.$$

By scaling back to the original time scale, $m(s(t))$ has the same value for

$$\{t, \phi(t), \phi^2(t), \ldots, \phi^n(t), \ldots\}. \tag{4.6}$$

Since $\phi^n(t) \neq t$ for almost every t and for all $n \in N$, (4.6) is an infinite set for almost every t. Therefore, if $\phi^n(t) \neq t$, the set of (4.6) has at least one limit point, where $m(s(t))$ is a locally recurrent function. If we define L as a set of points where $m(s(t))$ is locally recurrent, $m(s(L))$ is of measure zero since $m(s(t))$ has finite derivative [10]. Furthermore, if we define K as a set of points such that $\phi^n(t) = t$ for some $n \in N$, $m(s(K))$ is of measure zero since K is of measure zero. Since the range of $m(s(t))$ is the same with $m(s(L)) \cup m(s(K))$, the range of $m(s(t))$ is also of measure zero, or $m(s(t))$ is a constant function (zero function since $m(0) = 0$). Therefore, $\phi^n(t) \neq t$ for almost every t and for all $n \in N$ implies that a nontrivial function m cannot satisfy (4.1) or (4.3).

(Only if). If the measure of fixed points is not zero or $\mu\{t \mid \phi^n(t) = t\} \neq 0$, and assuming that $n \in N$ is the minimal value for which the measure of the set is nonzero, we can find a closed interval $A_1 = [\alpha, \beta] \subset \mu\{t \mid \phi^n(t) = t\}$ since $\phi^n(t)$ is smooth except at a finite number of points. We now show that this implies the existence of m and n as in (4.1). Since $\phi^n(A_1) = A_1$,

$$A_1 \xrightarrow{\hat{r}} B_1 \xrightarrow{\hat{s}} A_2 \xrightarrow{\hat{r}} B_2 \cdots \xrightarrow{\hat{s}} A_{n-1} \xrightarrow{\hat{r}} B_{n-1} \xrightarrow{\hat{s}} A_1. \tag{4.7}$$

We can always make $r(t)$ and $s(t)$ monotone over A_i by reducing the size of A_1 (see Figure 4.1). Similarly, we can always reduce the size of A_1 to ensure that there is no intersection between the images of $r(t)$ over A_i and A_j unless $i = j$. Similarly, we can also ensure that the images of $s(t)$ over A_i and A_j do not intersect unless $i = j$. We now show that over these regions A_i, we can find $m(s(t))$ and $n(r(t))$ satisfying (4.1) or $m(s(t)) = n(r(t))$. Choose any nonzero smooth function $n(r(t))$ over A_1 such

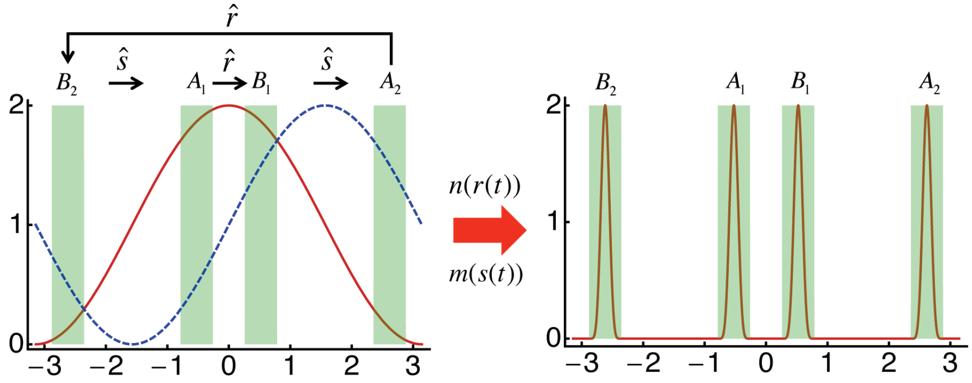


FIG. 4.1. *Description of the construction of* $n(r(t))$ *and* $m(s(t))$ *such that* $n(r(t)) = m(s(t))$ *when* $A_1 \subset \{t \mid \phi^2(t) = t\}$.

that $\frac{d^k n(r(t))}{dt^k} = 0$ for all k at the boundary of A_1 (Figure 4.1). This function can be extended over A_1^C as below:

$$n(r(t)) = \begin{cases} 0 & \text{if } t \notin A_i \text{ and } t \notin B_i, \\ n(r(\phi^{-(i-1)}(t))), & t \in A_i \text{ for } i = 2, 3, \ldots, n-1, \\ n(r(\phi^{-(i-1)}(\hat{r}(t)))), & t \in B_i \text{ for } i = 1, 2, \ldots, n-1. \end{cases} \tag{4.8}$$

$m(s(t))$ can also be chosen to match $n(r(t))$:

$$m(s(t)) = \begin{cases} 0 & \text{if } t \notin A_i \text{ and } t \notin B_i, \\ n(r(t)), & t \in A_i \text{ for } i = 1, 2, \ldots, n-1, \\ n(r(\hat{s}(t))), & t \in B_i \text{ for } i = 1, 2, \ldots, n-1. \end{cases} \tag{4.9}$$

Thus $m(s(t)) = n(r(t))$, and f and g are not unique by Theorem 4.1. ☐

Theorems 4.3 and 3.1 indicate that nonuniqueness rarely occurs since the equality of (3.2) must hold over a nonmeasure zero set, $\{t \mid \phi^n(t) = t\}$. Moreover, Theorem 4.3 provides a way to test whether f and g of (1.1) are unique. If there exists a nonmeasure zero set where $\phi^n(t) = t$ for some $n \in N$, f and g will not be unique if they exist (Figure 2.1). If the measure of all fixed points is zero, and f and g of (1.1) exist, f and g will be unique (Figure 2.1).

5. Sufficient conditions for existence and uniqueness of (1.2) and (1.3).

Here, we show that both existence and uniqueness can be achieved in the broader class of timecourses by considering another form of ODE models (1.2). Our previous study found that for smooth timecourses $r(t)$ and $s(t)$, (1.2) could be constructed if $dr/dt = 0 \Rightarrow d^2r/dt^2 \neq 0$ [3]. That is, for this class of functions $r(t)$, f and g of form (1.2) exist. Here, we show that f and g of form (1.2) are also unique. To show uniqueness, we need a different proof for the existence of (1.2) from the original proof [3], which is described in Theorem 5.2.

LEMMA 5.1. *Given smooth τ-periodic even timecourse $a(t)$ and odd timecourse $b(t)$, unique smooth functions p and q exist such that*[1]

$$q(\cos(2\pi t/\tau)) = a(t), \tag{5.1}$$

$$dp(\cos(2\pi t/\tau))/dt = b(t). \tag{5.2}$$

THEOREM 5.2. *Given smooth τ-periodic timecourses $r(t)$ and $s(t)$, unique smooth functions f and g of* (1.2) *exist if $dr/dt = 0 \Rightarrow d^2r/dt^2 \neq 0$.*

Proof. Without loss of generality, let us assume that $r(t)$ has a maximum at $t = 0$. A vertical shift and scaling of $r(t)$ can make its range $[-1, 1]$. Then, by scaling time we can make $r(t') = \cos(2\pi t'/\tau)$. This scale is smooth because both dt/dt' and dt'/dt are well defined:

$$\frac{dt}{dt'} = \frac{dr/dt'}{dr/dt} = \frac{-(2\pi/\tau)\sin(2\pi t'/\tau)}{dr/dt}. \tag{5.3}$$

If $dr/dt = 0$, then $\sin(2\pi t'/\tau) = 0$ and $d^2r/dt^2 \neq 0$. Therefore, (5.3) is well defined by l'Hôpital's rule. In the same way, dt'/dt is also well defined. Furthermore, $dt/dt' \neq 0$ and $dt'/dt \neq 0$ imply

$$dt/dt' > 0 \text{ and } dt'/dt > 0. \tag{5.4}$$

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[1] This follows from arguments presented in [3].

Decomposition of $s(t')$ into its odd and even parts such that

$$s(t') = s(t')_{odd} + s(t')_{even} \tag{5.5}$$

allows the form

$$s(t') = dp(r(t'))/dt' + q(r(t')) \tag{5.6}$$

because we can find smooth functions q and p such that $s(t')_{even} = q(r(t'))$ and $s(t')_{odd} = dp(r(t'))/dt'$ by Lemma 5.1. Now by scaling back to the original time, we can get

$$s(t) = dp(r(t))/dt' + q(r(t)). \tag{5.7}$$

But we need $dr(t)/dt$ instead of $dr(t)/dt'$. For this, let us consider the ratio of $dr(t')/dt'$ and $(dr(t')/dt)_{odd}$:

$$\begin{aligned}\frac{dr/dt'}{(dr/dt)_{odd}} &= \frac{2dr(t')/dt'}{dr(t')/dt - dr(-t')/dt}\\ &= \frac{2}{(dr(t')/dt)/(dr(t')/dt') - (dr(-t')/dt)/(dr(t')/dt')}.\end{aligned}$$

Since $dr(t')/dt'$ is an odd function,

$$\frac{dr/dt'}{(dr/dt)_{odd}} = \frac{2}{dt'(t')/dt + dt'(-t')/dt}. \tag{5.8}$$

The denominator of (5.8) is never zero due to (5.4). Therefore, (5.8) is well defined and smooth. Furthermore, (5.8) is an even function because it is the ratio of two odd functions. Therefore, with Lemma 5.1, we can find smooth function f_a such that

$$\frac{dr/dt'}{(dr/dt)_{odd}} = f_a(r). \tag{5.9}$$

We can also find a smooth function f_b such that

$$\frac{dr(t')}{dt_{even}} = f_b(r(t')), \tag{5.10}$$

$$\frac{dr}{dt_{even}}(t') = f_a(r)\frac{dr}{dt}_{odd} = f_a(r)\left(\frac{dr}{dt} - \frac{dr}{dt}_{even}\right) = f_a(r)\left(\frac{dr}{dt} - f_b(r)\right). \tag{5.11}$$

From this,

$$\begin{aligned} s(t) &= \frac{dp(r(t))}{dt'} + q(r(t)) = \frac{dp}{dr}\frac{dr}{dt'} + q(r) = \frac{dp}{dr}f_a(r)\left(\frac{dr}{dt} - f_b(r)\right) + q(r)\\ &= \frac{dp}{dr}f_a(r)\frac{dr}{dt} - \frac{dp}{dr}f_a(r)f_b(r) + q(r) = \frac{df(r)}{dr}\frac{dr}{dt} - g(r) = \frac{df(r)}{dt} - g(r),\end{aligned} \tag{5.12}$$

where $df(r)/dr = (dp/dr)f_a(r)$ and $g(r) = f_a(r)f_b(r)(dp/dr) - q(r)$.

Now, let us show the uniqueness of f and g. Let $s(t) = df_1(r)/dt - g_1(r) = df_2(r)/dt - g_2(r)$:

$$\begin{aligned} \frac{df_1}{dt} - g_1(r) &= \frac{df_2}{dt} - g_2(r), \\ \frac{df_1}{dr}\frac{dr}{dt} - g_1(r) &= \frac{df_2}{dr}\frac{dr}{dt} - g_2(r), \\ \frac{df_1}{dr}\left(\frac{dr}{dt'}\frac{1}{f_a(r)} + f_b(r)\right) - g_1(r) &= \frac{df_2}{dr}\left(\frac{dr}{dt'}\frac{1}{f_a(r)} + f_b(r)\right) - g_2(r). \end{aligned} \tag{5.13}$$

Note that since (5.9) is nonzero, $1/f_a(r)$ is well defined. With the time scale t', $r(t')$ is an even function and $dr(t')/dt'$ is an odd function. Let us take the odd part of (5.13):

$$\frac{df_1}{dr}\frac{dr}{dt'}\frac{1}{f_a(r)} = \frac{df_2}{dr}\frac{dr}{dt'}\frac{1}{f_a(r)}. \tag{5.14}$$

Equation (5.14) implies $df_1/dt' = df_2/dt'$, which means $df_1/dt = df_2/dt$. Then, $g_1(r) = g_2(r)$ follows. ☐

Unfortunately, it is difficult to interpret the biological meaning of f and g of (1.2). Now, we propose a way to convert (1.2) into a more common form of the model so that f and g of (1.2) can be interpreted as production and degradation rates.

THEOREM 5.3. *Given smooth* τ*-periodic timecourses* $r(t)$ *and* $s(t)$*, smooth functions* f *and* g *of* (1.3) *exist uniquely if* $dr/dt = 0 \Rightarrow d^2r/dt^2 \neq 0$, $ds/dt = 0 \Rightarrow d^2s/dt^2 \neq 0$, *and*

$$\phi(t) = \hat{s}(\hat{r}(t)) \neq t \text{ for all } t. \tag{5.15}$$

Proof. Let us show that dp/dr of (5.6) is well defined and not zero for all t. From (5.5) and (5.6),

$$s(t') = s(t')_{odd} + s(t')_{even} = \frac{dp(r(t'))}{dt'} + q(r(t')) = \frac{dp}{dr}\frac{dr}{dt'} + q(r(t')), \tag{5.16}$$

$$\frac{dp}{dr} = \frac{s(t')_{odd}}{dr/dt'} = \frac{s(t')_{odd}}{(dr/dt)(dt/dt')}. \tag{5.17}$$

Since $dr/dt \neq 0$ except for extrema ($t' = 0$ and $-\tau/2$ on $[-\tau/2, \tau/2)$) and $dt/dt' \neq 0$ from (5.4), dp/dr is well defined for $t' \neq 0$ and $t' \neq -\tau/2$. If $s(t')_{odd}$ has zero except for $t' = 0$ and $t' = -\tau/2$, then $s(t') = s(t')_{even}$ or $\phi(t') = \hat{s}(\hat{r}(t')) = t'$ because $r(t')$ is an even function, which contradicts (5.15). Therefore, $s(t')_{odd}$ is not zero except for $t' = 0$ and $t' = -\tau/2$ on $[-\tau/2, \tau/2)$. Therefore, $dp/dr \neq 0$ except for $t' = 0$ and $t' = -\tau/2$. Furthermore, for $t' = 0$ and $t' = -\tau/2$,

$$\frac{dp}{dr}_{t'=0,\tau/2} = \frac{s_{odd}(t')}{dr(t')/dt'} = \frac{ds_{odd}(t')/dt'}{dr^2(t')/dt'^2} = \frac{ds(t')/dt'}{(dr^2(t')/dt^2)(dt/dt')^2}. \tag{5.18}$$

The second equality comes from l'Hôpital's rule, and the third equality comes from $ds_{even}(t')/dt' = 0$ at $t' = 0$ and $-\tau/2$. Since $dt/dt' \neq 0$ from (5.4) and $dr^2/dt^2 \neq 0$ at $t' = 0$ and $-\tau/2$, dp/dr is well defined. Furthermore, $ds(t')/dt' \neq 0$ at $t' = 0$ and $-\tau/2$ because $s(t')$ does not have an extremum at $t' = 0$ and $-\tau/2$ by (5.15). This implies that $dp/dr \neq 0$ at $t' = 0$ and $-\tau/2$. Therefore, dp/dr is well defined and nonzero for all t' or t. From (5.8) and (5.9), $f_a(r)$ is well defined and not zero for all t. Therefore, (5.12) can be modified to

$$\begin{aligned} s(t) &= \frac{dp}{dr} f_a(r) \frac{dr}{dt} - \frac{dp}{dr} f_a(r) f_b(r) + q(r) = \frac{dp}{dr} f_a(r) \frac{dr}{dt} - h(r), \\ \frac{dr}{dt} &= f(r)s(t) - g(r), \end{aligned}$$

where $f(r) = 1/(f_a(r)dp/dr)$ and $g(r) = -h(r)/(f_a(r)dp/dr)$. $\square$

Note that (1.3) is a canonical type of model with a production rate and degradation rate. The difference between (1.3) and (1.1) is that the production rate depends on both $r(t)$ and $s(t)$.

6. Conclusion. Our results show how mathematical models could be constructed given two oscillating timecourses, $r(t)$ and $s(t)$ from a biochemical system. Models of the form (1.1) have perhaps the greatest biological intuition, but also lack existence and uniqueness. Models of form (1.2) can be broadly constructed, and are also unique, but their biological interpretation is limited. Models of form (1.3) contain properties in between models of forms (1.1) and (1.2). Interestingly, most results were obtained by studying the fixed points of an iterating map $\phi^n(t)$. Future work should study these iterative maps to more easily determine their fixed points. Additionally, our assumption about complete knowledge of the timecourse data is unrealistic. Given the noisy nature of all biological data, statistical methods should be incorporated with our methods to estimate f and g or rule them out. We hope our results will encourage consideration of well-posedness in the construction of models of biological timekeeping.

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